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A s-myly Route toward Lymphoid Differentiation

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In this issue of *Immunity*, Ng et al. (2009) show that lymphoid-lineage priming occurs in hematopoietic stem cells and is dependent on the Ikaros transcription factor, as is repression of self-renewal genes during lymphoid differentiation.

Hematopoietic stem cells (HSCs) have extensive self-renewal potential and the ability to differentiate into at least eight distinct cell lineages. Despite intense investigation, our understanding of how HSCs choose a differentiated fate is still in its infancy. Many studies have focused on defining precursor-progeny relationships through use of cell surface markers for precursor cell isolation combined with in vitro or in vivo differentiation assays to retrospectively examine developmental potential. Such assays led to the identification of a common lymphoid progenitor (CLP) and a common myeloid progenitor (CMP, which has myeloid, megakaryocyte [Mk], and erythrocyte [E] potential) and to the notion that the initial fate choice of HSCs was between lymphoid and myeloid differentiation (Wagers et al., 2002). Recent segregation of the HSC population (Lineage marker negative, *c-kit*$^+$, Sca1$^+$) via surface expression of the Flt3 receptor led to identification of a lymphoid-primed multipotent progenitor (LMPP), which retains lymphoid and myeloid differentiation potential but has a substantially reduced capacity for Mk and E differentiation. Therefore, LMPPs appears to represent an intermediate stage between HSCs and CLPs, indicating that MkE developmental potential is lost prior to segregation of the lymphoid and myeloid fates (Luc et al., 2008). In this issue of *Immunity*, Ng et al. (2009) provide molecular evidence supporting this model of hematopoiesis and identify the Ikaros transcription factor as a regulator of the lymphoid gene signature in multipotent progenitors.

Gene expression analysis in HSCs and their multipotent progeny has been consistent with the developmental potential exhibited by these cells. For example, mRNA transcripts associated with mature lymphoid and myeloid cells are detected at low amounts in LMPPs, a process referred to as ''lineage priming'' (Mansson et al., 2007). Indeed, CLPs show evidence of lymphoid- but not myeloid-lineage priming whereas CMPs have myeloid- but not lymphoid-lineage priming (Akashi et al., 2003). Lineage priming may be the consequence of alterations in chromatin structure that allow increased accessibility of these genes to their transcriptional regulators and/or an increase in expression of the appropriate set of transcription factors. Because mRNA transcripts associated with multiple lineages have been detected in a single cell, further lineage restriction appears to involve the resolution of these conflicting gene expression programs through repression of inappropriate genes and/or reinforcement of lineage-appropriate genes. Importantly, to date, MkE- and myeloid-lineage priming have been detected in HSCs; however, lymphoid-lineage priming has been detected only at the LMPP stage (Luc et al., 2008). This skewed lineage priming in HSCs has been interpreted to mean that the molecular events underlying the lymphoid fate choice are initiated late in differentiation, possibly reflecting the late evolution of the lymphoid lineages.

Ng et al. (2009) identify transcriptional signatures in HSCs and their multipotent progeny that reveal a very early onset of multilineage, including lymphoid, priming and provide evidence for the current view of lineage relationships through transcriptome-based ''phylogenetic'' analysis. Global gene expression analysis was used to compare the transcriptomes of populations enriched for HSCs, LMPPs, GMPs, and MEPs and clustering identified signatures that are unique or common between sets of these progenitors, for example a stem-myeloid-lymphoid (s-myly) signature containing genes common between HSCs, GMPs, and LMPPs or a differentiated lymphoid (d-ly) signature containing genes found only in LMPPs and pro-B lymphocytes. Through this analysis, a set of lymphoid-associated mRNA transcripts was identified that is already primed in the HSC population. An elegant single-cell analysis of gene expression was undertaken to determine the extent of lymphoid-lineage priming in HSCs and the degree of overlap between lymphoid-, myeloid-, and eyrthroid-lineage priming as well as with stem cell signature genes. This analysis revealed a similar frequency of HSCs with lymphoid- or erythroid-lineage priming, suggesting that these lineages are primed with equal efficiency in HSCs and concurrently with the expression of genes involved in HSC self-renewal. However, only a small fraction of HSCs was found to have copriming of lymphoid, myeloid, and erythroid mRNA transcripts and these were equally divided between cells with or without the stem cell signature (presumably representing true HSCs and MPPs, respectively), suggesting that lineage priming is a stochastic process.

This study also revealed that the subset of GMPs lacking the myeloid surface marker Mac1 and expressing an Ikaros promoter-GFP reporter expresses both myeloid and lymphoid mRNA transcripts, even though GMPs are considered to be

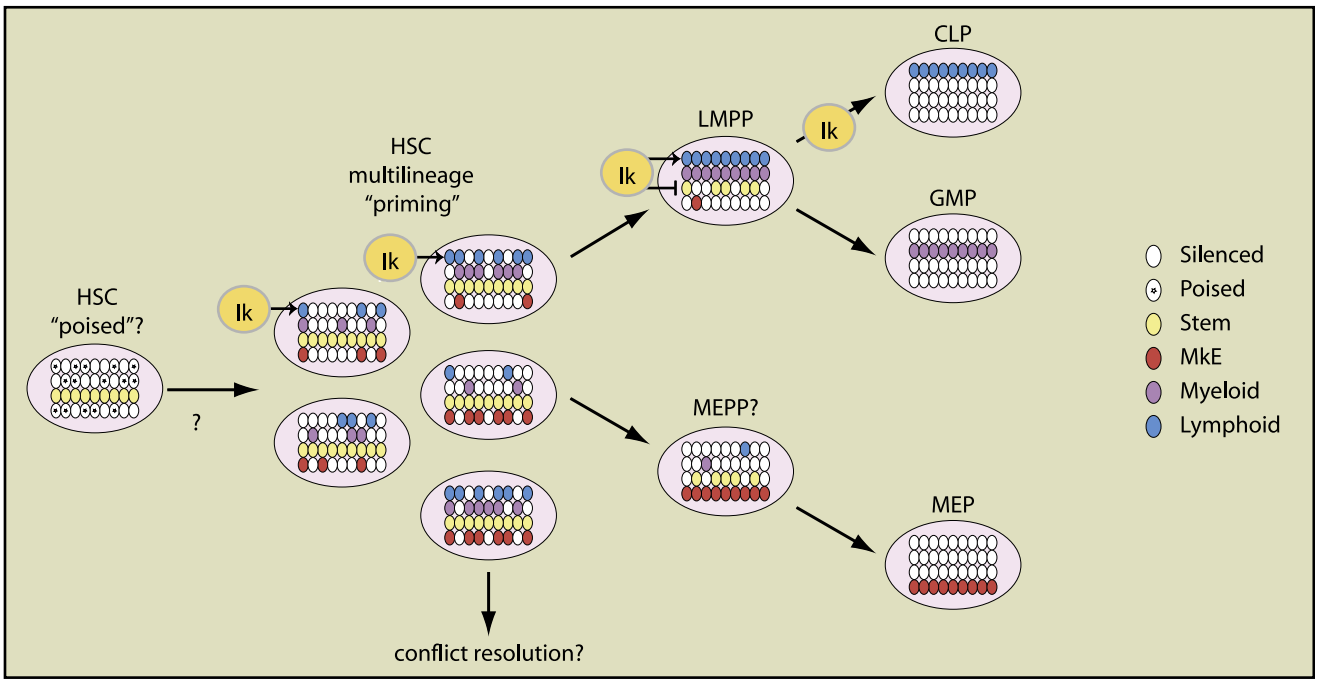

Figure 1. Modeling HSC Differentiation Based on Patterns of Lineage Priming
HSCs stochastically transcribe low amounts ("priming") of genes associated with multiple lineages. An immature subset of HSCs may have these genes in a "poised" chromatin state. As HSCs differentiate, expression of stem cell genes declines and cells with lineage-restricted priming are likely to adopt the corresponding fate; the fate of cells that efficiently prime conflicting programs remains to be determined. MEPs express only MkE genes but a more immature MkE-primed progenitor (MEPP) may exist, with multilineage priming, that is closely related to HSCs. Ikaros (Ik) promotes lymphoid priming in HSCs and their lymphoid leaning progeny and prevents expression of stem cells genes in LMPPs. Ikaros is required for proper lymphoid specification beyond the LMPPs, suggesting that lymphoid priming is important for these later cell fate decisions.

myeloid-restricted progenitors. Indeed, the authors show that these cells give rise to both B and T lymphocytes after in vivo transfer, indicating that they retain lymphoid in addition to myeloid differentiation potential. However, quantitative in vitro assays revealed that these GMPs efficiently produce T lymphocytes but are less robust at producing B lymphocytes. This finding suggests that B lymphocyte potential can be lost before myeloid or T lymphocyte potential and is consistent with recent experiments that revealed T lymphocyte and myeloid, but not B lymphocyte, potential in the earliest thymic progenitors (Chi et al., 2009). Therefore, these experiments demonstrate that a subset of GMPs, which are likely an immature subset recently derived from LMPPs, retain a latent lymphoid (T > B) potential as they undergo restriction to the myeloid lineage.

Ng et al. (2009) performed a parallel single-cell gene expression analysis in HSCs and their multipotent progeny isolated from mice lacking the transcriptional regulator Ikaros. Ikaros-deficient HSCs and LMPPs showed a decrease in lymphoid-lineage priming and an increase in multiple mRNA transcripts associated with HSC self-renewal. Because Ikaros is both a transcriptional activator and repressor, the authors conclude that Ikaros is a bivalent regulator of cell fate, activating lymphoid-lineage-associated genes and repressing HSC-associated genes. However, direct repression of this subset of HSC genes by Ikaros remains to be demonstrated. It is interesting that a subset of lymphoid mRNA transcripts that are Ikaros dependent also depend on the transcription factor E2A, which is required for development of LMPPs and for lymphoid-lineage priming (Dias et al., 2008). Because many of these lymphoid mRNA transcripts have putative binding sites for both E2A and Ikaros in their promoter, it is likely that these transcription factors function cooperatively to initiate lymphoid-lineage priming. However, additional transcription factors may also function in this process in a combinatorial manner.

This paper provides important insight into the hierarchical relationships between very early progenitors based on patterns of lineage priming but also raises a number of interesting questions about hematopoietic cell fate decisions (Figure 1). Does lineage priming occur equally well in long-term (LT) and short-term (ST) reconstituting HSCs or is lineage priming associated with the decision of LT-HSCs to differentiate? In embryonic stem cells it has been demonstrated that some genes are "poised" to be expressed by containing histone modifications associated with both active and repressed chromatin (Spivakov and Fisher, 2007). These bivalent modifications are proposed to allow rapid activation or repression of a gene based on the availability of transcriptional regulators. It remains to be determined whether a subset of HSCs maintains hematopoietic lineage-associated genes in such a poised state or whether these genes are in a repressed state and transcription is initiated only after significant chromatin remodeling. If hematopoietic genes are poised in LT-HSCs, then lineage priming may occur rapidly in the presence of the correct complement of transcription factors. Alternatively, the need to remodel chromatin may delay the activation of

these genes until later stages of differentiation. In either scenario, the complement of transcription factors, rather than lineage priming itself, may play a direct role in HSC cell fate determination. However, the two models may be distinguished by whether lineage priming occurs concomitant with or subsequent to cell fate decisions.

In this regard, the role of lineage priming in segregation of the LMPP and MkE fates remains to be resolved. Because Ikaros is not required for development of LMPPs but rather for lymphoid differentiation from LMPPs, a possible role for lymphoid-lineage priming appears to be restricted to cell fate decisions after segregation of the MkE fate (Ng et al., 2009; Yoshida et al., 2006). However, it remains possible that a critical Ikaros-independent lymphoid gene(s) may function in repression of the MkE fate. It is also unclear whether HSCs that coprime lymphoid- and myeloid- along with erythroid-lineage genes resolve these conflicting gene expression programs or whether they simply fail to undergo further differentiation (Figure 1). Although the degree of multilineage copriming appears to be low in this study, this may be due to the small number of genes examined. Therefore, analysis of a larger set of lineage-associated genes may reveal a higher degree of copriming and a need for resolution of conflicting gene expression programs at this stage. Acquisition of the LMPP fate could be the consequence of HSC differentiation concomitant with a failure of MkE-lineage priming (i.e., those cells that fail to activate MkE genes become LMPPs). In this respect, it is interesting that one of the primed E lineage genes examined is Gata1, a transcription factor that is essential for E development whereas none of the essential lymphoid transcription factors are a component of the lymphoid-lineage-primed set (Crispino, 2005). The ability to analyze chromatin and global gene expression patterns in single cells is a challenging future goal that will be required to understand how genome regulation influences cell fate choices. The identification of s-myly or other multilineage gene programs in progenitors with defined developmental potential is an important step in understanding how multiple lineages arise from HSCs.

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Fighting the Flu with Inflammasome Signaling

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A wide variety of stimuli induce the inflammasome, but little is known about its role in immune protection against viruses. In this issue of *Immunity*, Allen et al. (2009) and Thomas et al. (2009) describe a critical role for NLRP3 induction of the inflammasome and protection against influenza virus infection.

Influenza A virus is an important human pathogen that infects millions of people worldwide in seasonal epidemics and leads to more than 30,000 deaths annually in the United States alone (Taubenberger and Morens, 2008). The character of the immune response, and in particular the innate immune response, is a key determinant of influenza outcome wherein innate immunity mediates our essential first-line defense against infection. Pathogen-associated molecular patterns (PAMPs) present within influenza A virus that are generated during infection are recognized by three major classes of pattern-recognition receptors (PRRs), which form the basis for innate immune detection of viruses and other microbes. These PRRs include the Toll-like receptors (TLRs), retinoic acid inducible gene-I (RIG-I)-like receptors (RLRs), and the nucleotide-binding domain-leucine-rich repeat-containing molecules (NLRs). Detection of influenza A virus by TLRs or RLRs lead to the production of type 1 interferons in bronchial epithelial cells (via RIG-I) and plasmacytoid dendritic cells (via TLR7) leading to tissue-specific and systemic antiviral states (Wang et al., 2007). In general, little is known